

Dear team Munich,

Last week I performed a halo experiment to test if *S. aureus* has sensitivity to subtilin, produced by *B. subtilis*. This was performed in the ML-II lab of the MolGen group at the University of Groningen and supervised by Renske van Raaphorst. Below I report what steps are taken and give an overview of the results. Also I provide you with some references of the strains we used.

**Methods:**

30-09-2014

- Inoculated three different strains of *S. aureus* (see below) in 3 ml Nutrient Broth (Difco). Also two different *B. subtilis* strains were inoculated under different conditions (see below). The ATCC6633 strain was inoculated in 3 ml LB and in 3 ml of the defined medium SMM. Also the 168 strain was inoculated in 3 ml LB. These samples were grown overnight at 37°C in a shaking incubator.

Used strains *S. aureus*:

(All strains were provided by Auke van Heel)

1. CAL (MRSA obtained at the UMCG hospital)
2. NWZ (MRSA obtained at the UMCG hospital)
3. CECT240 (*Staphylococcus aureus* subsp. *aureus*, Rosenbach 1884)

Used strains *B. subtilis*:

1. ATCC6633 LB
2. ATCC6633 SMM (Preparation of SMM (Spizizen, 1958) with trace elements (Harwood and Archibald, 1990))
3. 168 LB

01-10-2014

- Of all the *S. aureus* strains two different blood agar plates were made. The first set was named CAL1, NWZ1, CECT1, which meant that per plate 200 µl of sheep blood was added to 10 ml Colorado Blood agar. These were poured in different plates and afterwards 120 µl of each *S. aureus* strain was streaked out on top of it.
- The second set was named CAL2, NWZ2, CECT2. In these plates 200 µl of sheep blood was added to 10 ml Colorado Blood agar per plate. These were poured on top of 120 µl of each *S. aureus* strain on each plate separately. This was mixed thoroughly so that the agar was mixed with the strain.
- All the plates were dried under the fumehood.
- On these six plates 6 µl of the three different sets of *B. subtilis* were spotted and put at 37 °C for 24h.

02-10-2014

- All the diameters of the halo's were measured and the plates were discarded. (See results below).

**Results:**

*S. aureus* plated (labeled #1):

CAL1: no results (see Fig. 1)

NWZ1: no results (see Fig. 2)

CECT1: no results (see Fig. 3)

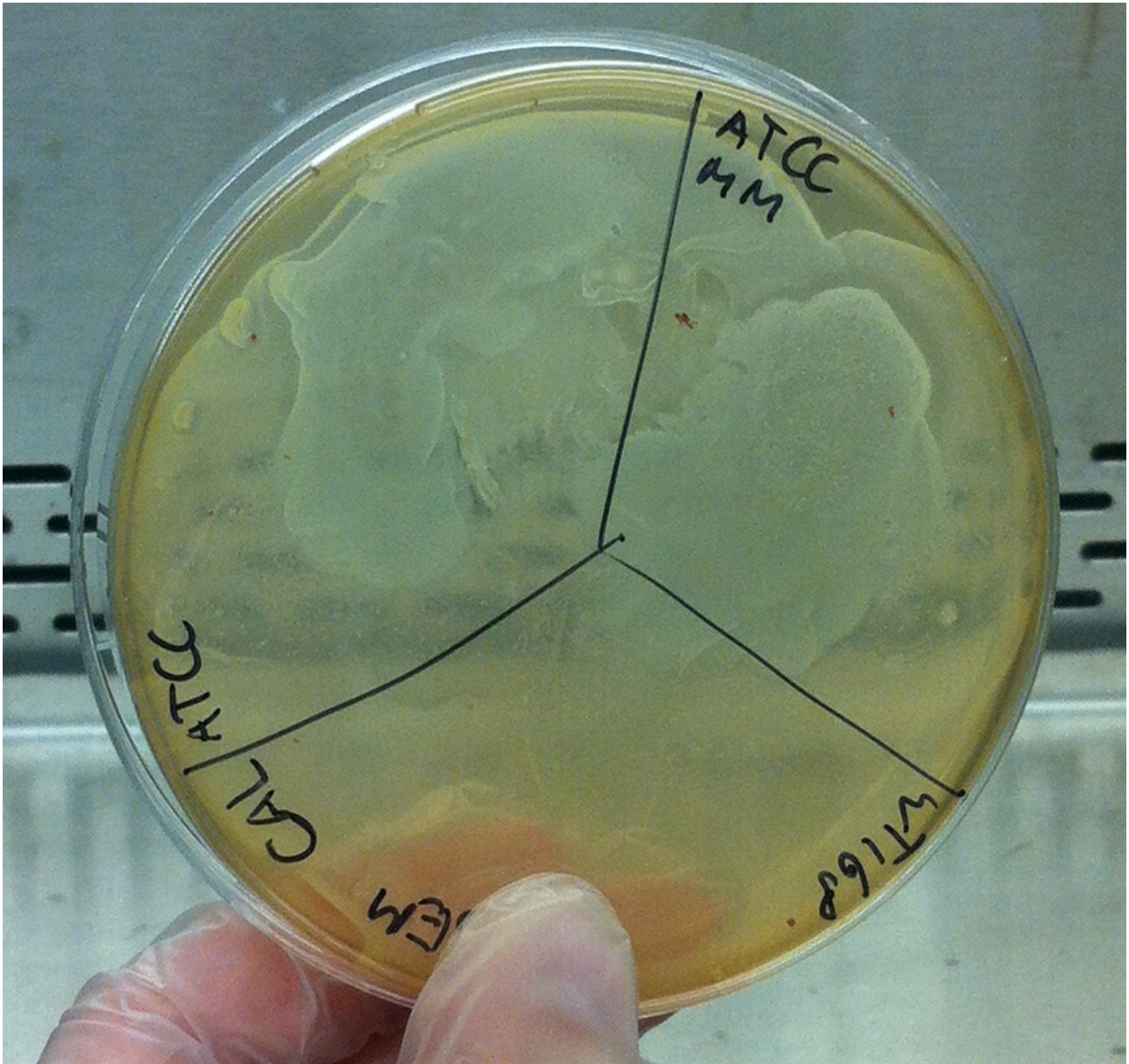


Fig. 1: CAL1 spotted with *B. subtilis* 168, ATCC6633 grown in LB and ATCC6633 grown in SMM.



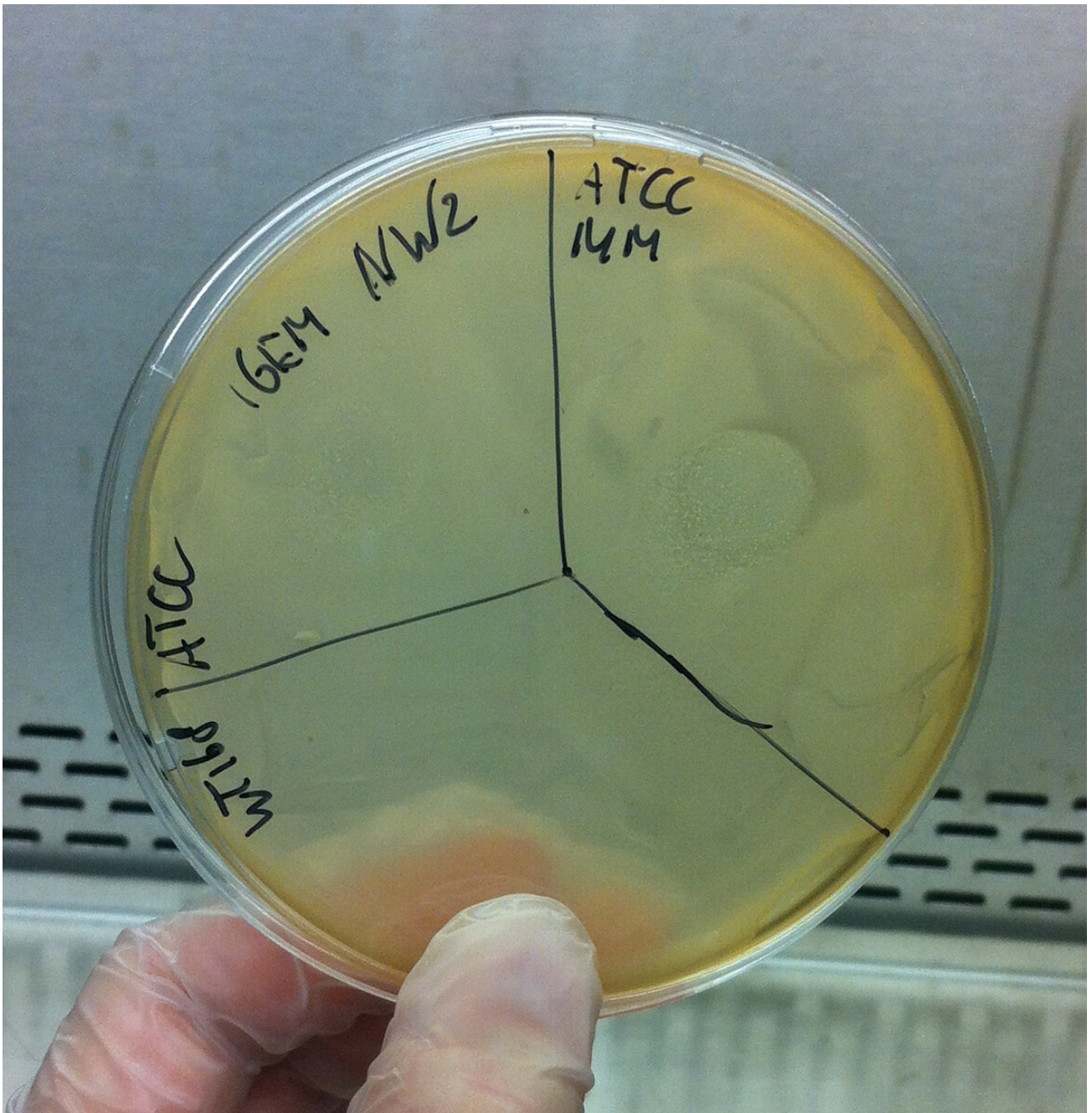


Fig. 2: NWZ1 spotted with *B. subtilis* 168, ATCC6633 grown in LB and ATCC6633 grown in SMM.



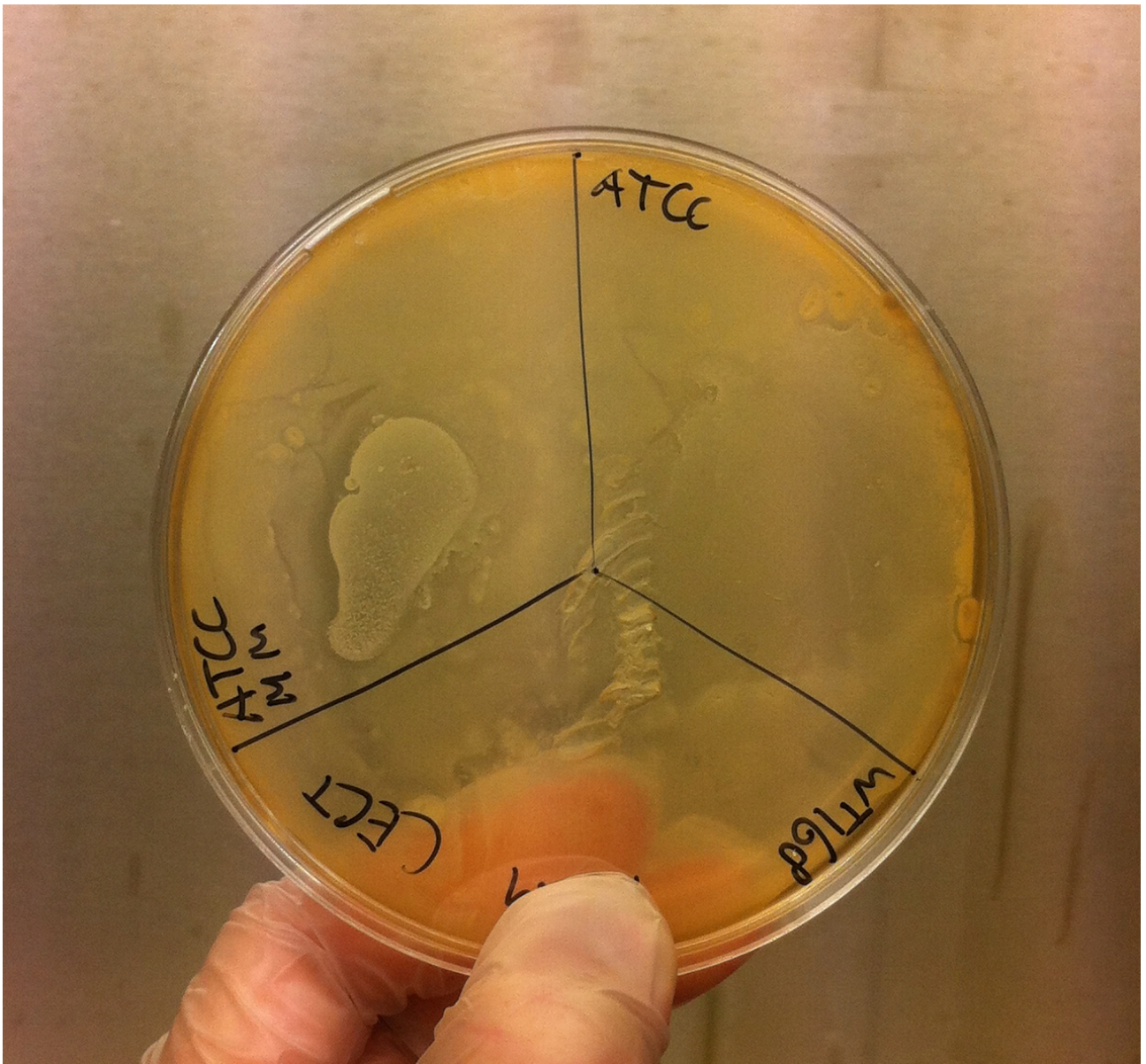


Fig. 3: CECT1 spotted with *B. subtilis* 168, ATCC6633 grown in LB and ATCC6633 grown in SMM.



*S. aureus* in agar (labeled #2):

CAL2/ATCC (Fig. 4)

colony: 4,0 mm

colony with halo: 6,0 mm

halo: 2,0 mm

CAL2/ATCC SMM (Fig. 4)

colony: 4,0 mm

colony with halo: 6,0 mm

halo: 2,0 mm

CAL2/168 (Fig. 4)

colony: 0 mm

colony with halo: 0 mm

halo: 0 mm

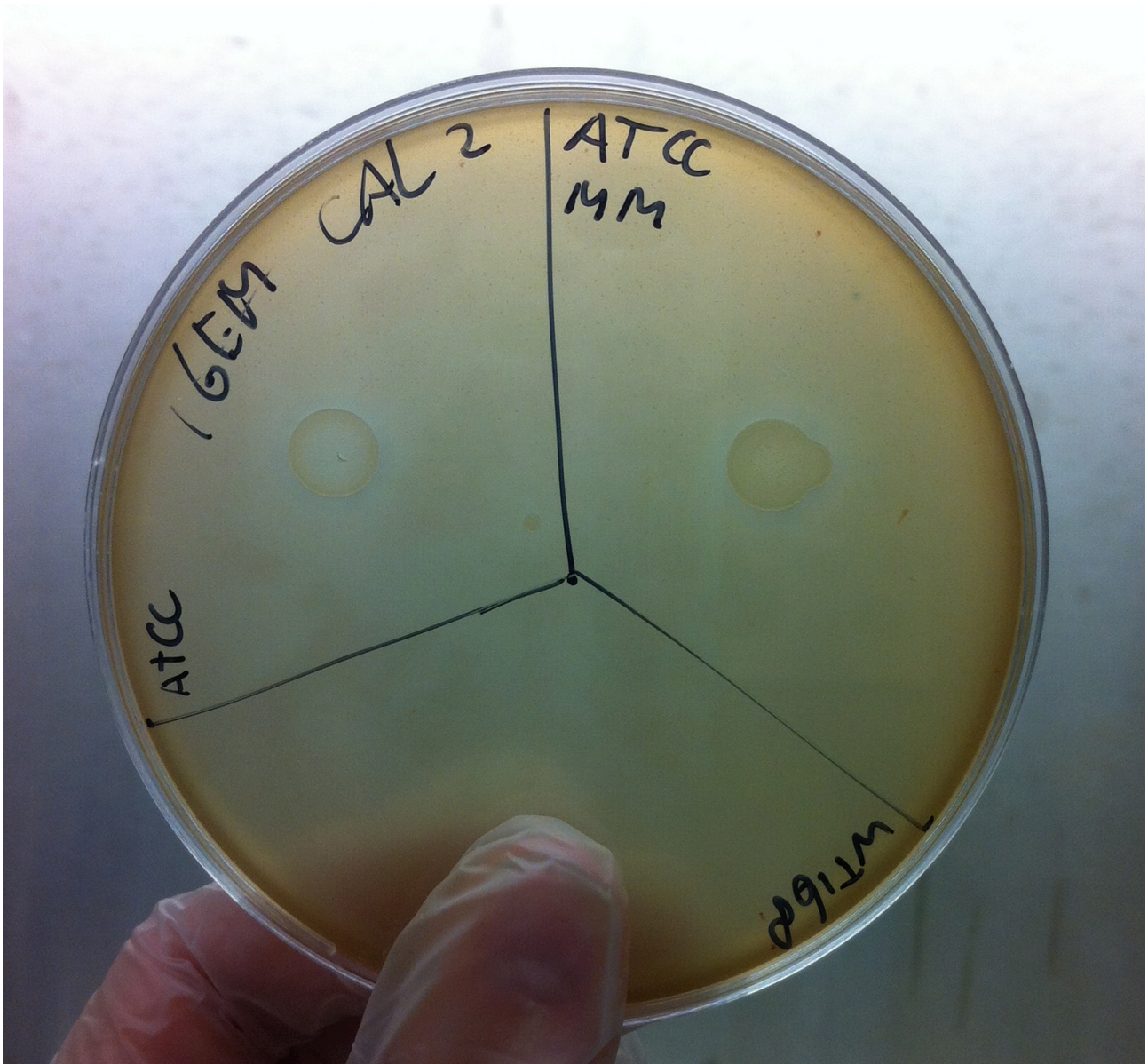


Fig. 4: CAL2 spotted with *B. subtilis* 168, ATCC6633 grown in LB and ATCC6633 grown in SMM.



NWZ2/ATCC (Fig. 5)

colony: 3,0 mm

colony with halo: 6,0 mm

halo: 3,0 mm

NWZ2/ATCC SMM (Fig. 5)

colony: 3,0 mm

colony with halo: 5,0 mm

halo: 2,0 mm

NWZ2/168 (Fig. 5)

colony: 0 mm

colony with halo: 0 mm

halo: 0 mm

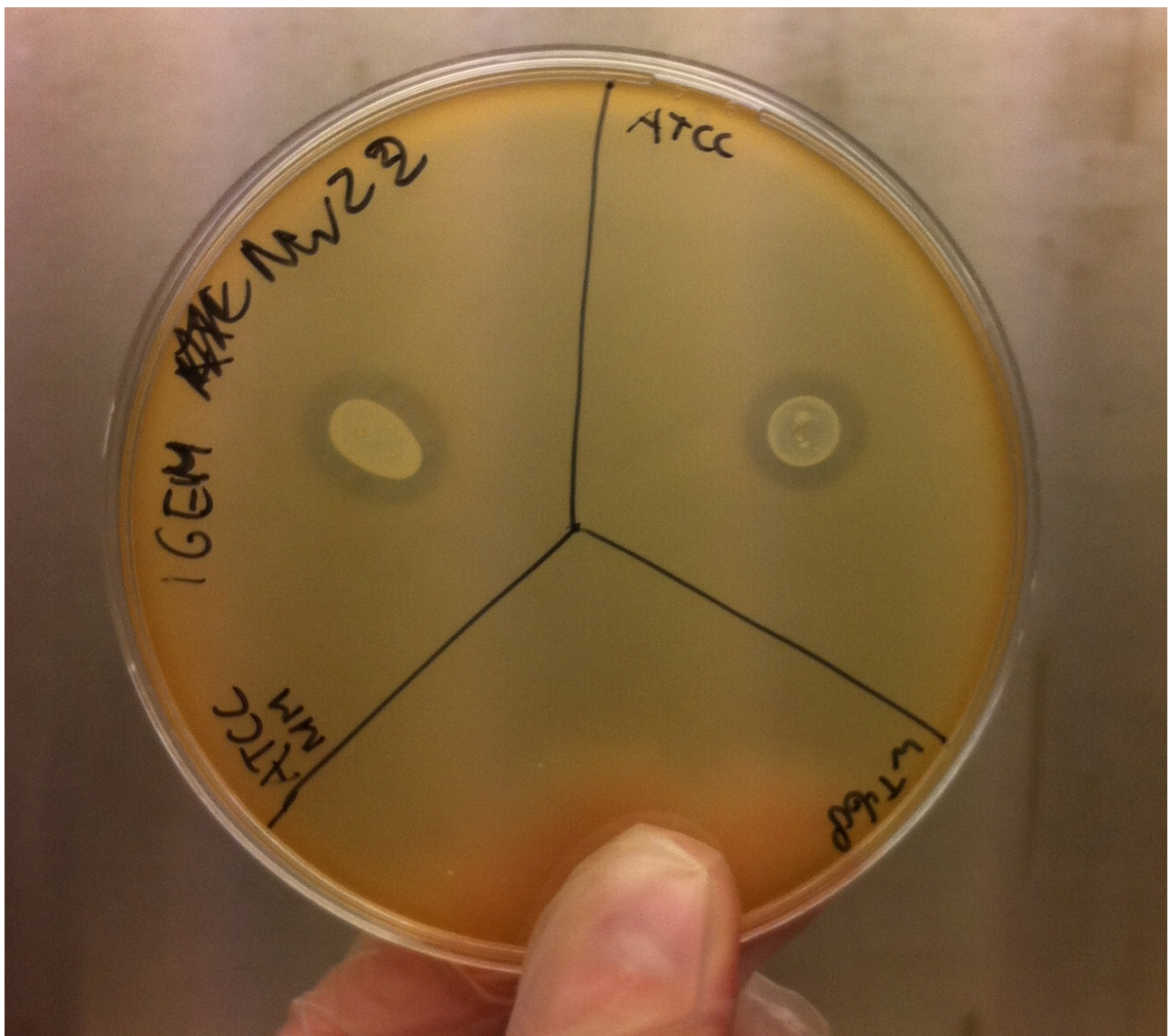


Fig. 5: NWZ2 spotted with *B. subtilis* 168, ATCC6633 grown in LB and ATCC6633 grown in SMM.



CECT2/ATCC (Fig. 6)

colony: 3,5 mm

colony with halo: 6,0 mm

halo: 2,5 mm

CECT2/ATCC SMM (Fig. 6)

colony: 3,5 mm

colony with halo: 6,5 mm

halo: 3,0 mm

CECT2/168 (Fig. 6)

colony: 0 mm

colony with halo: 0 mm

halo: 0 mm

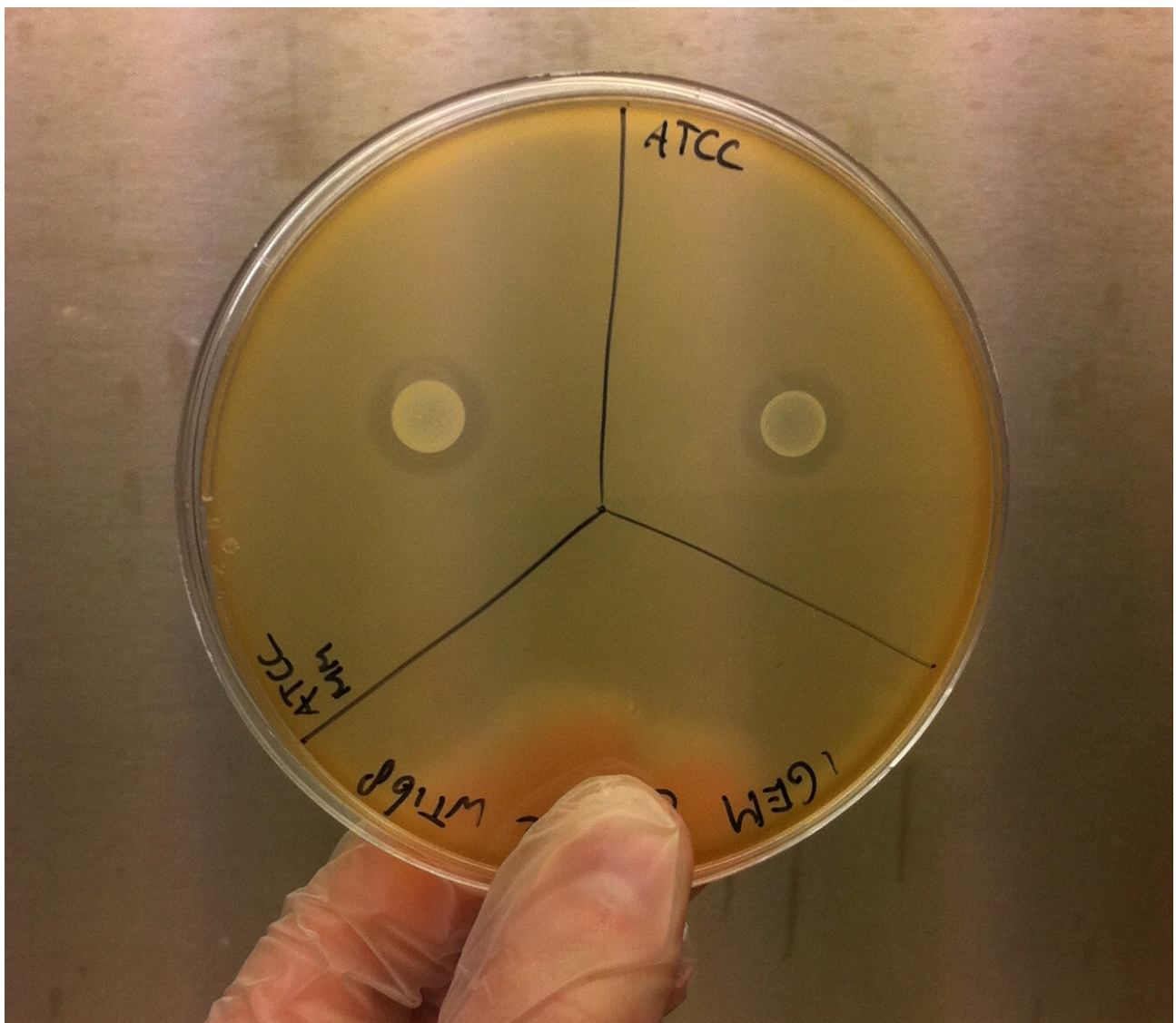


Fig. 6: CECT2 spotted with *B. subtilis* 168, ATCC6633 grown in LB and ATCC6633 grown in SMM.